ACS Pharmacology & Translational Science

pubs.acs.org/ptsci

Viewpoint

Can Papain-like Protease Inhibitors Halt SARS-CoV-2 Replication?

Biplab K Maiti*

Cite This: *ACS Pharmacol. Transl. Sci.* 2020, 3, 1017−1019

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: SARS-CoV-2 encoded papain-like protease (PLpro) harbors a labile Zn site (Cys_{189}−X−X−Cys_{192}−X_n−Cys_{224}−X−Cys_{226}) and a classic catalytic site (Cys_{111}−His_{272}−Asp_{286}), which play key roles for viral replication and hence represent promising drug targets. In this Viewpoint, both sulfur-based drugs and peptides-based inhibitors may block Cys residues in the catalytic and/or Zn site of CoV-2-PLpro, leading to dysfunction of CoV-2-PLpro and thereby halting viral replication.

KEYWORDS: *COVID-19, SARS-CoV-2 replication, papain-like protease, Zn-Cys_4, cysteine−drug complex*

Coronavirus disease 2019 (COVID-19) is a global pandemic illness, which is caused by the silent outbreaks of novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and thereby rapid knocks down worldwide health and economy.[1] Currently without a properly registered drug/therapy, COVID-19 is out of control, resulting in the loss of many lives. Therefore, it has raised serious concerns regarding the measures available to control this emerging viral disease. Hence, it is a major challenge to discover the proper drug/therapy that can markedly slow the spread of SARS-CoV-2.

Viral infection of the host is associated with virus life cycle including attachment, entry, replication, transcription, translation, maturation, release, and so on.[2] By inhibiting any one step in the life cycle, virus invasion can be halted. At the initial stage, SARS-CoV-2 plugs in to a human surface receptor protein, human angiotensin-converting enzyme 2 (hACE2), prior to entry into the host cell.[3] The interruption of virus−host cross-talk is one potential therapeutic intervention which can halt viral infection.[4] The next step, upon entry into the host cell, SARS-CoV-2 releases two viral cysteine proteases, papain-like protease (PLpro) and the 3-chymotrypsin-like protease (3CLpro), which are critically important for viral replication.[5] Therefore, these proteases are potential targets for the development of anti-CoV-2 drugs which may stop viral replication and thereby reduce the outbreak of SARS-CoV-2. However, currently, information on CoV-2 PLpro is relatively rarer than that available on CoV-2 3CLpro. Therefore, this Viewpoint sheds light on a thiol-targeting CoV-2 PLpro inhibitor that may block conserved Cys residues in a putative Zn site and/or the catalytic site of CoV-2 PLpro, leading to dysfunction of CoV-2 PLpro and thereby impeding the progression of viral replication.

Currently, the crystal structure[6] of CoV-2 PLpro is available and reveals that CoV-2 PLpro harbors three domains similar to those in CoV PLpro:[5] (1) catalytic cysteine cleavage domain, (2) putative labile Zn-binding domain, and (3) ubiquitin domain (Figure 1).[6] The catalytic site represents a classic amino acid triad, Cys_{111}−His_{272}−Asp_{286}, which plays a key role for viral replication. In the Zn site, the Zn^{II} ion is labile and tetrahedrally coordinated by four conserved Cys residues (Cys_{189}−X−X−Cys_{192}−X_n−Cys_{224}−X−Cys_{226}), and is also essential for catalysis because it holds the structural integrity of CoV-2 PLpro. Furthermore, PLpro has an additional domain, a ubiquitin domain that triggers the host's innate immune responses.[6] Therefore, all the domains in a single protein, CoV-2 PLpro, are likely to be promising therapeutic targets, but herein we discuss the labile Zn site as well as the catalytic site which are directly involved in viral replication.

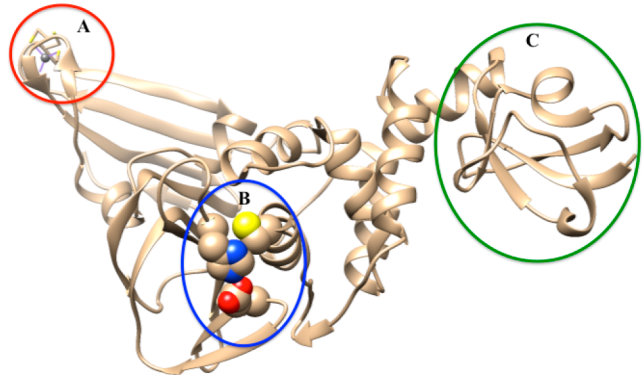

Figure 1. Crystal structure of SARS CoV-2-PLpro (Protein Databank (PDB) 6W9C)[6] represents three domains: (A) putative labile Zn-binding domain; Zn (gray ball) is coordinated with four Cys residues (C_{189}, C_{192}, C_{224}, and C_{226} are shown by stick), (B) catalytic domain (Cys_{111}−His_{272}−Asp_{286} triad is shown by sphere with color code; C: light gray, O: red, N: blue, and S: yellow), and (C) ubiquitin domain.

In viral PLpro, the conserved cysteine residue serves two roles: (1) structural role by binding the Zn^{II} ion (that induces the correct protein folding and stabilizes the local geometry) and (2) catalytic role by nucleophilic attack on substrate, leading to

Received: July 23, 2020
Published: August 4, 2020

the structure/function of PLpro. Therefore, ejection of Zn^{II} ion from the putative labile Zn site and/or blocking of the Cys residue at the catalytic site of CoV-2 PLpro are potential antiviral therapies which may lead to dysfunction of CoV-2 PLpro and thereby retard viral replication. However, no registered drugs or therapies agents targeting CoV-2 PLpro are on the market yet. Using the above guidelines, the initial step is to identify potential thiol-reacting inhibitors from marketed drugs, which can covalently bind conserved Cys residues in CoV-2-PLpro to yield a Cys−inhibitor complex[7] (Figure 2), which ultimately

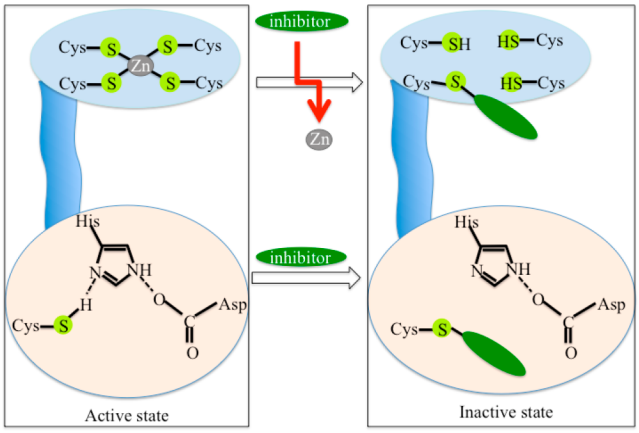

Figure 2. Cartoon representing the possible mechanism of SARS-CoV-2 PLpro (Zn site (light sky blue) and catalytic site (light orange)) with inhibitor (green oval).

inactivates the CoV-2-PLpro activity, resulting in interference of the virus replication. Various categories of inhibitors as Zn-ejectors are reported. The most common category of Zn-ejectors is soft electrophiles, disulfide-based thiol-reacting inhibitors such as disulfiram (DSF). DSF, a clinically safe, FDA-approved drug to treat chronic alcohol dependence, has been extensively studied in cell cultures against other viruses, including HIV-1, HCV, MERS, and SARS, for ejection of Zn^{II} from PLpro.[8,9] As both CoV-2-PLpro and CoV-PLpro share ∼83% of their genome sequence[10] and have similar structures,[6] DSF may likely to be considered as a potential Zn-ejector drug against CoV-2-PLpro.[7] Recently, Sargsyan et al.[11] reports that DSF shows dual functions, Zn-ejector as well as cysteine modifier drug, against CoV-2 PLpro, leading to the loss of structure/function of CoV-2-PLpro and thereby inhibiting virus replication in vitro. This result raises interest in the use of other sulfur-based drugs against COVID-19 illness. Varieties of sulfur-based FDA-approved drugs are in line for testing of COVID-19, such as diethyldithiocarbamate (DDC), captopril, 2,2′-dithiodipyridine, 2,2′-dithiobis(benzothiazole), 6-thioguanine (6-TG), and so on. For instance, Swaim et al.[12] report that 6-TG strongly inhibits the enzyme activity of CoV-2-PLpro and thereby halts CoV-2 replication in cell cultures at submicromolar concentrations. In addition, a metallothiol-based drug, auranofin, an FDA-approved drug to treat rheumatoid arthritis, can strongly inhibit replication of COV-2 in human cells, even at low micromolar levels.[13]

The SARS family encodes two large multidomain polyproteins must be cleaved into 16 nonstructural proteins (nsps) for viral RNA synthesis by two cysteine proteases (PLpro and 3CLpro).[5] PLpro recognizes a consensus cleavage motif, LXGG (X = any amino acid, L = leucine, and G = glycine), which is present between two nsps in polyproteins such as nsp1 / nsp2, nsp2 / nsp3, and nsp3 / nsp4, and cleaves the peptide bond to release nsp1, nsp2, and nsp3 proteins which are key components for viral replication.[5] Therefore, small peptides as substrate-based inhibitors of CoV-2-PLpro enzyme may capable of unraveling COVID-19 illness. A handful of rationally designed small peptides as substrate-based inhibitors of PLpro of SARS family viruses have been developed and exhibit high selectivity toward PLpro binding, resulting in interference of viral replication.[14] Recently, Rut et al.[15] reported two tetrapeptides as CoV-2 PLpro inhibitors which strongly inhibit viral replication in vitro and cell lines: Ac-Abu(Bth)-Dap-Gly-Gly-VME (VIR250) and Ac-hTyr-Dap-Gly-Gly-VME (VIR251) (hTyr: homotyrosine, Dap: diaminopimelic acid, Abu(Bth): 2-amino-3-(2′-benzothiazolyl)butyric acid, and VME = vinylmethyl ester; at positions, P4 and P3 in LXGG motif (P4−P1) are substituted by unnatural amino acids, hTyr/Abu(Bth) and Dap). The Gly residue at the P1 position in the tetra-peptide is conjugated with VME, which facilitates thioether linkage with the catalytic Cys_{111} residue of CoV-2-PLpro, leading to inactivation of the polyprotein cleavage activity of CoV-2-PLpro and thereby halts the replication of CoV-2. In addition, VIR251 has more potent efficacy but relatively lower selectivity toward CoV PLpro binding than does VIR250. This seems to be due to the different orientation of hTyr with respect to Abu(Bth) in the catalytic site. The crystal structure of CoV-2-PLpro-bound VIR251 (or VIR250, not shown here) complex provides a complete map of the active site with inhibitor (Figure 3),[15] which may guide the design and development of substrate-based anti-COVID-19 drugs with potent efficacy and high selectivity.

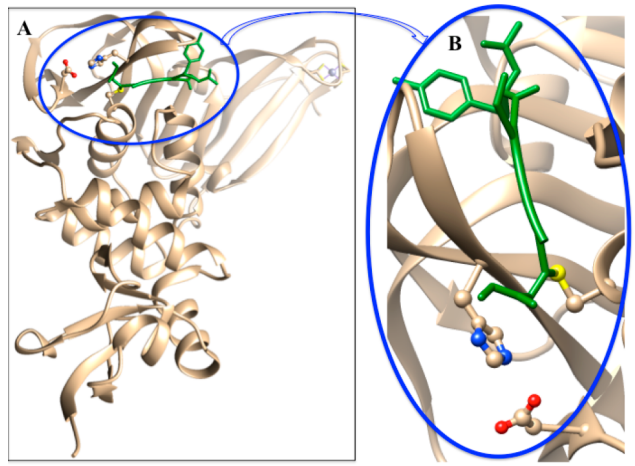

Figure 3. Crystal structure of SARS CoV-2-PLpro-bound VIR251 complex (PDB 6WX4)[15] showing (A) the thioether linkage between inhibitor (stick model with green color) and Cys_{111} residue (ball-and-stick model with color code; C: light gray and S: yellow) in catalytic site (magnified and highlighted by blue oval (B)).

Overall, SARS-CoV-2 PLpro represents as an attractive drug target for development of anti-CoV-2 drugs. Putative labile Zn sites are also found in other subdomains such as nsp3, nsp10, and nsp13, which would be also promising drug targets.[7] A Zn-ejector drug has lower selectivity, because it may remove Zn^{II} not only from the target protein but also from other Zn-containing proteins in the human body. In this regard, a substrate-base inhibitor would be a better choice, because it can selectively bind the Cys residue at the catalytic site of CoV-2-

https://dx.doi.org/10.1021/acsptsci.0c00093
ACS Pharmacol. Transl. Sci. 2020, 3, 1017−1019

PLpro, arresting the progression of viral replication. The potent antiviral activity of a peptide-based inhibitor could be improved by rational design. Therefore, thiol-targeting inhibitor therapy would be a promising path for treatment of the recent outbreak of SARS-CoV-2.

AUTHOR INFORMATION

Corresponding Author

Biplab K Maiti − *National Institute of Technology Sikkim, Ravangla Campus, Barfung Block, Ravangla Sub Division, South Sikkim 737139, India;* orcid.org/0000-0002-0985-0031; Email: biplab@nitsikkim.ac.in

Complete contact information is available at: https://pubs.acs.org/10.1021/acsptsci.0c00093

Notes

The author declares no competing financial interest.

ACKNOWLEDGMENTS

The author thanks the National Institute of Technology, Sikkim, India.

REFERENCES

(1) Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., Tu, W., Chen, C., Jin, L., Yang, R., Wang, Q., Zhou, S., Wang, R., Liu, H., Luo, Y., Liu, Y., Shao, G., Li, H., Tao, Z., Yang, Y., Deng, Z., Liu, B., Ma, Z., Zhang, Y., Shi, G., Lam, T. T. Y., Wu, J. T., Gao, G. F., Cowling, B. J., Yang, B., Leung, G. M., and Feng, Z. (2020) Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med. 382*, 1199−1207.

(2) Ryu, W.-S. (2017) Virus Life Cycle. *Molecular Virology of Human Pathogenic Viruses*, 31−45.

(3) (a) Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., and Li, F. (2020) Structural basis of receptor recognition by SARS- CoV-2. *Nature 581*, 221−224. (b) Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., and Wang, X. (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature 581*, 215−220.

(4) Zhang, G., Pomplun, S., Loftis, A. R., Loas, A., and Pentelute, B. L. (2020) The first-in-class peptide binder to the SARS-CoV-2 spike protein, *bioRxiv.org e-Print archive*, https://www.biorxiv.org/content/10.1101/2020.03.19.999318.

(5) Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R., and Hilgenfeld, R. (2003) Coronavirus main proteinase (3CLpro) structure: Basis for design of anti-SARS drugs. *Science 300*, 1763−1767. (b) Baez-Santos, Y. M., St. John, S. E., and Mesecar, A. D. (2015) The SARS-coronavirus papain-like protease: Structure, function and inhibition by designed antiviral compounds. *Antiviral Res. 115*, 21−38.

(6) Michalska, K., Kim, Y., Jedrzejczak, R., Maltseva, N. I., Stols, L., Endres, M., and Joachimiak, A. (2020) The crystal structure of papain-like protease of SARS CoV-2. *Protein Databank*, https://www.wwpdb.org/pdb?id=pdb_00006w9c.

(7) Sargsyan, K., Chen, T., Grauffel, C., and Lim, C. (2020) Identifying COVID-19 Drug-Sites Susceptible To Clinically Safe Zn-ejector Drugs Using Evolutionary/Physical Principles, *OSF Preprints*, https://doi.org/10.31219/osf.io/snuqf.

(8) (a) Garg, D., and Torbett, B. E. (2014) Advances in targeting nucleocapsid-nucleic acid interactions in HIV-1 therapy. *Virus Res. 193*, 135−143. (b) Lee, Y.-M., Wang, Y.-T., Duh, Y., Yuan, H. S., and Lim, C. (2013) Identification of Labile Zn Sites in Drug-Target Proteins. *J. Am. Chem. Soc. 135*, 14028−14031.

(9) Lin, M.-H., Moses, D. C., Hsieh, C.-H., Cheng, S.-C., Chen, Y.-H., Sun, C.-Y., and Chou, C.-Y. (2018) Disulfiram can inhibit MERS and SARS coronavirus papain-like proteases via different modes. *Antiviral Res. 150*, 155−163.

(10) Xu, J., Zhao, S., Teng, T., Abdalla, A. E., Zhu, W., Xie, L., Wang, Y., and Guo, X. (2020) Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses 12*, 244−261.

(11) Sargsyan, K., Lin, C.-C., Chen, T., Grauffel, C., Chen, Y.-P., Yang, W.-Z., Yuan, H. S., and Lim, C. (2020) Multi-Targeting of Functional Cysteines in Multiple Conserved SARS-CoV-2 Domains by Clinically Safe Zn-ejectors, *ChemRxiv.org e-Print archive*, https://chemrxiv.org/articles/preprint/Multi-Targeting_of_Functional_Cysteines_in_Multiple_Conserved_SARS-CoV-2_Domains_by_Clinically_Safe_Zn-ejectors/12179037.

(12) Swaim, C. D., Perng, Y.-C., Zhao, X., Canadeo, L. A., Harastani, H. H., Darling, T. L., Boon, A. C. M., Lenschow, D. J., and Huibregtse, J. M. (2020) 6-Thioguanine blocks SARS-CoV-2 replication by inhibition of PLpro protease activities, *bioRxiv.org e-Print archive*, https://www.biorxiv.org/content/10.1101/2020.07.01.183020v1.

(13) Rothan, H. A., Stone, S., Natekar, J., Kumari, P., Arora, K., and Kumar, M. (2020) The FDA-approved gold drug Auranofin inhibits novel coronavirus (SARS-COV-2) replication and attenuates inflammation in human cells, *bioRxiv.org e-Print archive*, https://www.biorxiv.org/content/10.1101/2020.04.14.041228v1.

(14) Poreba, M., Salvesen, G. S., and Drag, M. (2017) Synthesis of a HyCoSuL peptide substrate library to dissect protease substrate specificity. *Nat. Protoc. 12*, 2189−2214.

(15) Rut, W., Lv, Z., Zmudzinski, M., Patchett, S., Nayak, D., Snipas, S. J., Oualid, F. E., Huang, T. T., Bekes, M., Drag, M., and Olsen, S. K. (2020) Activity profiling and structures of inhibitor-bound SARS-CoV-2-PLpro protease provides a framework for anti-COVID-19 drug design, *bioRxiv.org e-Print archive*, https://www.biorxiv.org/content/10.1101/2020.04.29.068890v2.